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Effect of N-ethylmaleimide on Stomatal Opening and Closing of *Commelina communis*

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Abstract

Effect of N-ethylmaleimide on stomatal opening and closing of *Commelina communis* was studied.

N-ethylmaleimide produced a strong inhibition both on stomatal opening in the light and closing in the dark. The addition of DTT to the bathing medium containing N-ethylmaleimide did not recover the N-ethylmaleimide-induced inhibition of stomatal opening, remaining closed stomata, but recovered the inhibition of stomatal closing and resulted in increasing stomatal closing.

It is suggested that both stomatal opening and closing are active processes, and ATPase transport system would play an important role on stomatal movement.

Introduction

It has been proposed by Fujino (1967) that stomatal opening and closing are based on the active transport of K^+ into and out of guard cells of *Commelina communis*, that is, ATP-ATPase system may be involved in the active transport.

Thereafter, it was convinced by many workers that stomatal opening is active process. However, there is only a few works about stomatal closing process.

Fujino (1967) reported that SH inhibitors, PCMB and iodoacetic acid increased stomatal opening of *Commelina communis*, though inhibit stomatal closing. Jinno and Fujino (1976) also reported same results from the experiment with ouabain.

On the other hand, Thomas (1970) indicated that stomatal opening was not affected by PCMB, but ouabain inhibited the short-term stomatal opening. Mouravieff (1970) reported that SH inhibitors N-ethylmaleimide, iodoacetamid and iodoacetic acid inhibited stomatal opening in *Veronica*, *Leucanthemum* and *Tradescantia*.

Thus, effects of SH inhibitors on stomatal movement are confusing. In this paper, the effect of N-ethylmaleimide and DTT both on stomatal opening and closing in *Commelina communis* will be described.

Abbreviation : DTT, dithiothreitol.

Materials and Methods

Commelina communis cultivated in a greenhouse was used in this experiment as materials.

Open samples : At about twelve on the day of experiment, entire leaves was excised and transferred to the experimental room, and was preexamined by a microscope to ensure that stomata were widely open. Leaves with open stomata (approximately 25μm) were used as open samples. When stomata did not open to about 25μm in width, leaves were floated on the water for about two hours under the light of about 8,000 lux at 30°C. Virtually most stomata opened to about 25μm in width.

Closed samples : On the evening of the previous day, entire leaves were excised and transferred to the experimental room, and kept floating on the water in an incubator at 30°C for one night, and resulted in closed stomata. By this pretreatment, when the stomata did not completely close, epidermal strips were immersed in distilled water for 30 min. Virtually most stomata completely closed.

Five abaxial epidermis, about 0.5×0.5cm, with the fully open stomata or completely closed stomata were immersed in 5ml of the bathing medium, and were kept in the dark or in the light of 8,000 lux for 4 hr respectively maintaing a temperature of 30°C. At least 20 stomata per each strip were measured at the middle of the strips by a microscope every one hour. Each value is an average of at least 100 individual stomatal aperture.

The incubation medium for stomatal opening was 60 mM phosphate buffer of pH 6.0 containg 75 mM KCl, while the medium for stomatal closure was 60 mM phosphate buffer of pH 6.0 without KCl.

To investigate the effect of N-ethylmalecmide on stomatal movement, 1, 0.1 and 0.01 mM at final concentration was applied to the control bathing medium. 1 mM DTT was applied to the bathing medium containg N-ethylmaleimide to investigate the effect on the N-ethylmaleimide-induced inhibition of stomatal movement.

Results

Fig. 1 shows the effect of the concentration of N-ethylmaleimide in bathing medium containing 75 mM KCl on the stomatal opening in the light. In the control medium, the stomatal opening in the light was progressively stimulated as the time proceeds. The extent of stomatal opening reduced as the concentration of N-ethylmaleimide increased. The presence of 1 mM N-ethylmaleimide strongly inhibited stomatal opening, and remained almost closed state. By the addition of 0.1 mM N-ethylmaleimide, stomatal opening was considerably inhibited. 0.01 mM N-ethylmaleimide had no significant effect on stomatal opening, showing almost similer rate of stomatal opening to that of control.

Fig. 2 shows the effect of N-ethylmaleimide on stomatal closing in bathing medium without KCl in the dark. Open stomata show rapid reduction of aperture in the control medium. The extent of stomatal closing reduced as the concentration of N-ethylmaleimide increased. The presence of 1 mM N-ethylmaleimide strongly inhibited stomatal closing in the dark and resulted in almost open state. At concentration of 1 mM considerable

inhibition occurred and progressive stomatal closure occured. Stomatal closing was not significantly affected by 0.01 mM, and resulted in almost similer rate of stomatal closing to that of control.

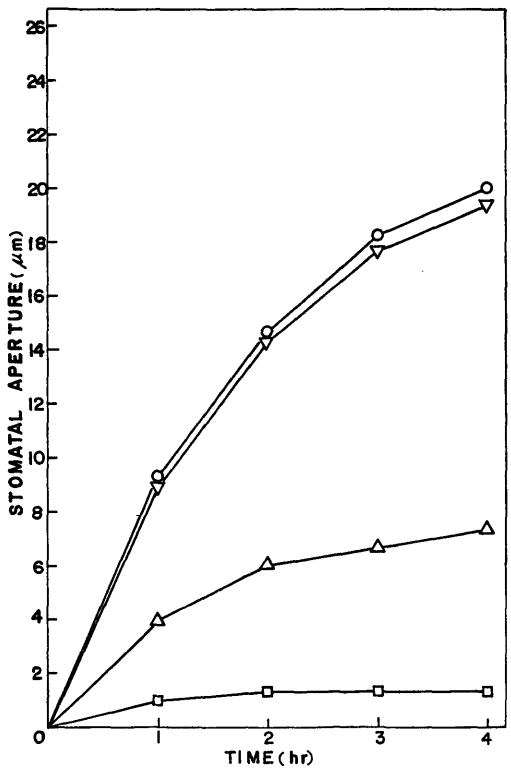

Fig. 1. Effect of N-ethylmaleimide on stomatal opening in the light. Closed samples were incubated in the control medium (○−○), 10^{-3}M (□−□), 10^{-4}M (△−△) and 10^{-5} M N-ethylmaleimide (▽−▽).

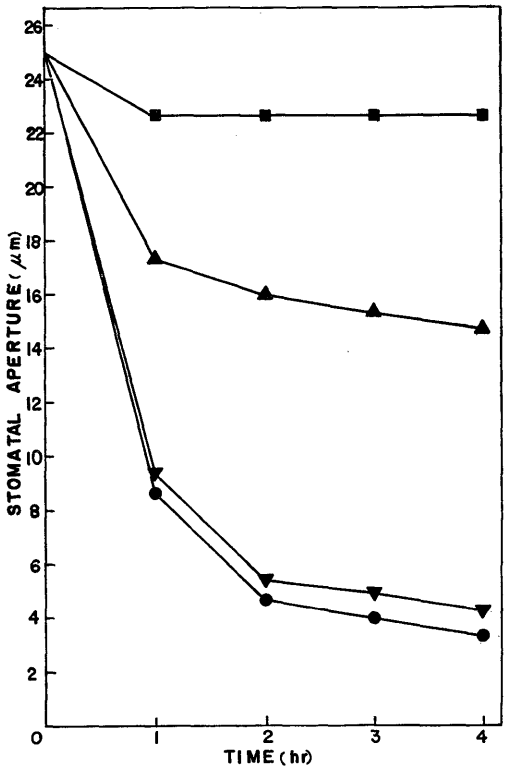

Fig. 2. Effect of N-ethylmaleimide on stomatal closing in the dark. Open samples were incubated in the control medium for closing (●−●), 10^{-3}M (■−■), 10^{-4}M (▲−▲) and 10^{-5}M N-ethylmaleimide (▼−▼).

Fig. 3 and 4 show the effect of the dithiothreitol on the N-ethylmaleimide-induced inhibition of stomatal movement. When 1 mM dithiothreitol was applied to 1 mM N-ethylmaleimide solution, stomatal opening in the light did not occurred, remaining almost closed stomata. On the other hand, stomatal closing was remarkably stimulated, producing a similer aperture to that in the control medium. That is, closing inhibition by N-ethylmaleimide was recovered by the application of dithiothreitol.

Discussion

If N-ethylmaleimide affects or alters the structural configuration of cell membrane, the permeability will increased and more faster stomatal closure will occur. However, the reverse result was obtained on stomatal closing in this experiment and then it seems

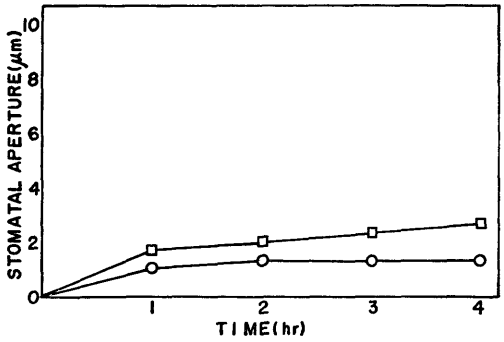

Fig. 3. Effect of DTT on N-ethylmaleimide induced-inhibition of stomatal opening in the light. ○—○ : 10^{-3}M N-ethylmaleimide ; □—□ : 10^{-3}M N-ethylmaleimide + 10^{-3}M DTT.

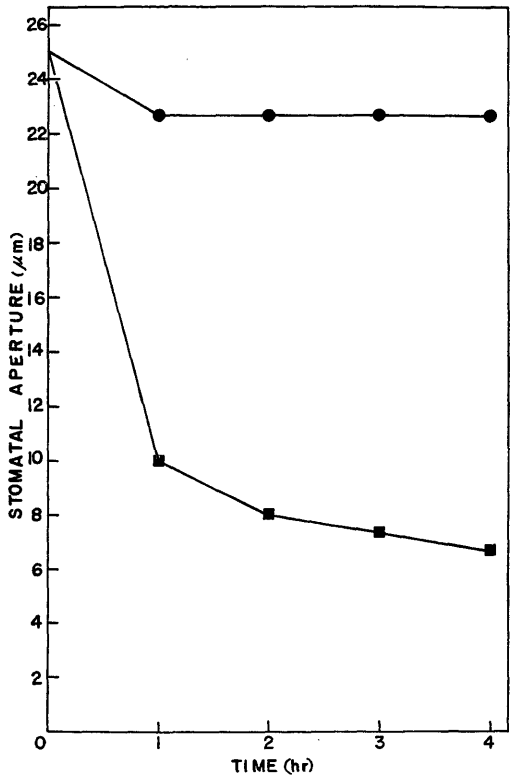

Fig. 4. Effect of DTT on N-ethymaleimide induced-inhibition of stomatal closing in the dark. ●—● : 10^{-3}M N-ethylmaleimide ; ■—■ : 10^{-3}M N-ethylmaleimide+10^{-3}M DTT.

that N-ethylmaleimide does not affect the membrane structure but the membrane-bound ATPase.

Present results strongly suggest that ATPase is involved in the stomatal movement and both stomatal opening and closing are active processes. That is, the inactivation of ATPase by addition of N-ethylmaleimide is considered to reduce the hydrolysis of ATP and result in less energy available to bring about the uptake of K^+ into the guard cells. The involvement of ATPase on stomatal closing is also suggested. The inactivation of ATPase is considered to reduce the hydrolysis of ATP bringing about the excretion of K^+ out of the guard cells.

Sulfhydryl group DTT is known to recover the effect of sulfhydryl inhibitor. It seems that the ATPase activity would be protected by the application of DTT in the dark. However, it is uncertain that the application of DTT had no effect on stomatal opening in the light. And then it seems that membrane-bound ATPase status or sensibility to the DTT in the light are different from that in the dark.

The result of N-ethylmaleimide application is in agreement with the observation of Mouravieff (1970) on stomatal opening of *Veronica*, *Leucanthemum* and *Tradescantia*.

On the other hand, the inactivation of ATP ase and the stimulation on stomatal opening of *Commelina communis* by the addition of PCMB (Fujino, 1967) and monoiodoacetic acid (Fujino, 1969) were reported. It was found that ouabain also stimulated stomatal opening of *Vicia faba* (Jinno and Fujino, 1975). While, it was considered that stomatal opening markedly affected by PCMB and ouabain inhibited the short-term stomatal opening (Thomas, 1970).

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